Protein Folding & Stability II

2303-Pos Board B73
Direct Characterization of Hydrophobic Hydration during Cold and Pressure Denaturation
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Many computational approaches for large biomolecular systems renormalize the role of the solvent into the definition of effective inter-residue interactions by coarse-grained models to access biologically relevant time-scales. However, the discrete nature of water and its effects on protein stability, dynamics and function cannot be explored in a predictive way with models that implicitly describe the solvent. We present here, a new off-lattice coarse-grained protein-like polymer model combined with a coarse-grained water model to correctly capture the solvent contribution in determining the stability of the collapsed state and molecular interactions. The water model includes tetrahedral interactions and correctly describes the phase diagram of water. The protein model exhibits pressure, cold and thermal denaturation. We will present the methodology and the results of how the length of a protein model and sequence of hydrophobic and hydrophilic monomers affects protein stability. We will show a mechanistic picture of how changes of hydrophobic hydration drive cold and pressure denaturation of proteins.

2304-Pos Board B74
Myoglobin. Addition of Chemical Details to the Coarse-Grained Model Enhances Resolution of Key-Features of RMS-Fluctuation in Monte-Carlo Simulated Data and Produces Universal Distribution of Amino-Acid Content into Four Transitional Groups
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Mechanism that is driving (un)folding transitions in a protein is one of the oldest biological devices ever built by evolution. Protein is a product of a DNA-RNA-Protein line. The mechanism of (un)folding must be universal. Protein reaches very high packing density during folding. These four conditions outline our side-chain backbone based Core-Shell model. The model develops the idea that the self-assembly takes place simultaneously at two - the peptide and side-chain, backbone levels. The Sliding Mechanism of (un)folding transitions developed in the Core-Shell model states, that under unfolding conditions, the rigid-peptide plane "softens" and the side-chain backbone performs transverse discrete moves across the peptide backbone. Sliding mechanism provides a residue's stereochemistry-specific score function that places the residue in one of four distinct Transitional groups.
Present work is a computational study of fluctuations of folded protein at near unfolding temperature. Our computational model of protein - a rigid-peptide plane model with Gô interactions, is simple enough to allow a step-by-step addition of details. We showed earlier, that there is a reasonable qualitative agreement between NMR, X-ray and the simplified model simulated data for small proteins. Here we show that a) though the simulation data is not accurate due to the lack of chemical details in the forcefield, the rms-fluctuation profile of simplified model Myoglobin detects better all key features of conformational fluctuations of native state protein; b) the addition of Ramachandran propensities improves resolution of computational key-features. The distribution of rms-fluctuation data into four Transitional Groups obtained for amino acid content of Myoglobin is strikingly similar to the distribution of 20 amino acids into Transitional groups - a proof on universality.

2305-Pos Board B75
Size, Shape and Motions of the SH3 Domain of the *Drosophila* Adapter Protein Drk
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The SH3 domain is a commonly found modular binding domain that functions to mediate protein-protein interactions and has been a frequent target of protein folding and other biophysical studies. The N-terminal SH3 domain (6.8 kDa) of the *Drosophila* adapter protein Drk is marginally stable under physiological conditions and exists in nearly 50/50 equilibrium between the folded and unfolded states, with a slow conversion rate of 2.2 s^{-1}(1). Due to the dynamic ensemble nature of this unfolded state under non-denaturing conditions it is difficult to characterize structurally. Experimental data have been used to define sets of heterogeneous conformations but ensemble averaging minimizes the information content (2). Importantly, little is known about inter-conversion rates within the unfolded ensemble.
Fluorescence correlation spectroscopy (FCS) experiments performed on the wild-type and on a mutant that stabilizes the folded state (T22G) provided the hydrodynamic radii of the folded ($R_{h,f}$), unfolded ($R_{h,u}$) and denatured ($R_{h,d}$) states at 20°C. Quite surprisingly, the results indicate that the unfolded state under non-denaturing conditions is less compact than the chemically-denatured state ($R_{h,u}/R_{h,f}$=1.31 ± 0.05 vs. $R_{h,d}/R_{h,f}$=1.21 ± 0.03). However, this can be explained by introducing adequate shape factors in previously reported NMR/SAXS data. In addition the FCS measured compactness of unfolded state is in an excellent agreement with PFG-NMR value of $R_{h,u}/R_{h,f}$=1.30 ± 0.01 (2). Furthermore, FCS analysis reveals a fast 200 ns process within the unfolded ensemble. To resolve slow (milli)second conformational dynamics, dual cysteine mutant (E2C-60C) was labelled with the Bodipy Fl/Alexa 647 FRET pair and single proteins were encapsulated in surface-tethered liposomes for long observations. This study provides further insights into the unfolded state heterogeneity and its importance in protein folding in general.
1.M Tollinger et al., J. Am. Chem. Soc.;123(46):11341-11352 (2001).
2.WY Choy et al., J. Mol. Biol.;316(1):101-112 (2002).

2306-Pos Board B76
Four-State Folding of a SH3 Domain: Salt-Induced Modulation of the Stabilities of the Intermediates and Native State
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Intermediates detected in the folding pathways of small proteins often serve as 'stepping stones', since they possess significant structural information. For many of the apparent two-state folders, it is merely a question of using the right folding conditions, or of using the right experimental probe, before a folding intermediate is revealed. The refolding of the PI3 kinase (PI3K) SH3 domain (previously assumed two-state, U ↔ N), had been shown to commence with the formation of a non-specific structure-less collapsed globule, U_C[1]. Kinetic folding studies were performed on this protein with the addition of a stabilizing co-solute to investigate the interplay of collapse and structure formation in the earliest steps of folding. A molten globule-like, on-pathway intermediate, L, stabilized by 500 mM Na_2SO_4, was detected within 6 ms of the initiation of folding. Both in the absence and presence of 500 mM Na_2SO_4, unfolding experiments also revealed an on-pathway native-like intermediate, M. The folding mechanism was therefore modelled using a minimal linear four-state mechanism (given below) which includes intermediates detected both before (U_C and L) and after (M) the rate-limiting step of the folding reaction. U ↔ (U_c ↔ L) ↔ M ↔ N Ongoing experiments aimed at elucidating the structural properties of L using tyrosine fluorescence, ANS fluorescence and multi-site FRET coupled to rapid mixing techniques indicate that L is a specific structural component of the collapsed ensemble in equilibrium with U_C. Similar experiments are also being performed to characterize the structural properties of the unfolding intermediate, M.
Reference:
1. Dasgupta, A. & Udgaonkar, J. B. (2010). Evidence for initial non-specific polypeptide chain collapse during the refolding of the SH3 domain of PI3 kinase. *J Mol Biol* **403**, 430-45.

2307-Pos Board B77
Hexafluoroisopropanol Induced Helix Sheet Transition of Stem Bromelain Correlation to Function
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Stembromelain is a proteolytic phytoprotein with a variety of therapeutic effects. Understanding its structural properties could provide insight into the mechanisms underlying its clinical utility. Stem bromelain was evaluated for its conformational and folding properties at the pH conditions it encounters when administered orally. It exists as a partially folded intermediate at pH 2.0. The conformational changes to this intermediate state evaluated using fluorinated alcohols known to induce changes similar to those seen in vivo. Studies using circular dichroism, fluorescence emission spectroscopy, binding of the hydrophobic dye 1-anilino-8-naphthalene sulfonic acid and mass spectrometry indicate that treatment with 10-30% hexafluoroisopropanol induces the partially folded intermediate to adoptmuchof the native protein's secondary structure, but only a rudimentary tertiary structure, characteristic of the molten globule state. Addition of slightly higher concentrations of hexafluoroisopropanol caused transformation from an a-helix to a b-sheet and induced formation of a compact nonnative structure. This nonnative form was more inhibitory of cell survival than either the native or the partially folded intermediate forms, as measured by enhanced suppression of proliferative cues and initiation of apoptotic events. The nonnative form also showed better antitumorigenic properties, as evaluated using an induced two-stage mouse skin

papilloma model. In contrast, the nonnative state showed only a fraction of the proteolytic activity of the native form. This study demonstrates that hexafluoroisopropanol can induce a conformational change in stem bromelain to a form with potentially useful therapeutic properties different from those of the native protein.

2308-Pos Board B78

Cold Denaturation in a Small Protein Domain

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Small, stable protein domains have become increasingly important as models for protein folding. However, one of the general thermodynamic characteristics of protein structures - cold denaturation - has not been observed for such model domains. We have investigated the thermal unfolding of a small 45 residue α-helical UBA domain using CD and fluorescence spectroscopy. In addition to a relatively high thermal stability ($T_m \sim 330K$), we have also detected unfolding at cold temperatures, whose onset begins around 285K in the absence of denaturant. To further probe the cold denaturation, urea was used to destabilize the protein and therefore shift the onset of the cold denaturation to higher temperatures. All experimental data could be explained using a simple thermodynamic model, which assumes linear dependence of the unfolding free energy (ΔG) on the denaturant concentration. The model yields a large positive heat capacity change upon unfolding, which is traditionally associated with solvent exposure of hydrophobic groups. This small UBA domain therefore provides a valuable model for studying the still controversial phenomenon of cold denaturation and for understanding folding of larger proteins, which exhibit cold denaturation behavior.

2309-Pos Board B79

Perturbing the Central Hydrophobic Cluster (CHC) of Aβ(10-35) by Incorporation of Fluorinated Phenylalanine Derivatives

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One of the putative causes of Alzheimer's disease involves aggregation of misfolded amyloid β (Aβ), a 39-42 residue polypeptide chain, and its subsequent deposition as amyloid plaques. The aggregation process proceeds via a nucleated polymerization mechanism where disordered peptide monomers interact with each other through hydrophobic interactions and rapidly extend and aggregate to eventually form larger fibrils with a highly ordered cross-strand β-sheet structure. It has also been suggested that the aromatic amino acid residues, tyrosine Y10 and phenylalanines (F19 and F20) in the central hydrophobic cluster (CHC) of the peptide play an important role in fibril assembly. In particular, F19 and F20 are suspected to be the drivers of the aggregation mechanism because of their hydrophobicity and aromaticity. In this context perturbation of the CHC through the introduction of non-natural (fluorinated) amino acids is expected to affect the aggregation process. Fluorinated amino acids in particular demonstrate distinct properties dictated by the presence of highly electronegative and hydrophobic fluorine atoms. However such fluorination is known to potentially eliminate the favorable interaction of aromatic hydrogens with the π-electron cloud, which can affect protein-protein interactions. In the present study the introduction of a pentafluoro-Phe in the hydrophobic core of the 26 residue Aβ peptide (Aβ10-35) and its effect on fibril formation has been investigated using circular dichroism (CD) and fluorescence methods which indicate a sequential conformational transition of the peptide from random coil → antiparallel β-sheets → parallel β-sheets. Transition time points have been obtained from these methods and compared to those obtained for the non-fluorinated peptide. UV resonance Raman (UVRR) studies have been performed to probe and characterize the vibrational modes of the fluoro-phenylalanines in the peptide and to explore their effect on the Phe-Phe π-stacking interactions.

2310-Pos Board B80

Kinetic Studies of the Monellin: Evidence for Switching Between Alternative Parallel Pathways

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To determine whether or not a protein uses multiple pathways to fold is an important goal in protein folding studies. When multiple pathways are present, defined by transition states that differ in their compactness and structure but not significantly in energy, they may manifest themselves by causing the dependence on denaturant concentration of the logarithm of the observed rate constant of folding, to have an upward curvature. Upward curvatures are normally not observable, but may become evident upon mutation if the mutation differentially destabilizes the transition states on the parallel pathways. Folding and unfolding kinetic studies performed with heterodimeric monellin (dcMN) and monomeric monellin (scMN), respectively, using the intrinsic tryptophan fluorescence of the protein as the probe, show chevron arms with upward curvatures. In this study, the folding mechanism of dcMN has been studied over a range of protein and guanidine hydrochloride (GdnHCl) concentrations. Folding is shown to occur in multiple kinetic phases. In the first stage of folding, which is silent to any change in intrinsic fluorescence, the two chains of monellin bind to one another to form an encounter complex. Interrupted folding experiments show that the initial encounter complex folds to native dcMN *via* two folding routes, and a productive folding intermediate is identified on one but not on both of these routes. The formation of the intermediate occurs in a fast kinetic phase, and its folding to native dcMN occurs in a slow kinetic phase. The folding chevron arms for both the fast and slow phases of folding are shown to have upward curvatures, suggesting that at least two pathways are operational during these kinetic phases of structure formation, and that folding switches from one pathway to the other as the GdnHCl concentration is increased.

2311-Pos Board B81

Direct Observation of Multistate Folding in a Single Beta-Helical Protein

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Filamentous haemagglutinin (FHA) is the major adhesin of B. pertussis, the bacterium that causes whooping cough. It is the prototypical member of the Two-Partner Secretion pathway family, a class of proteins associated with virulence in Gram-negative bacteria. Such proteins are large yet efficiently exported across the bacterial outer membrane without an obvious energy source, suggesting the hypothesis that translocation is driven by folding. Here, we use magnetic tweezers to apply stable and constant forces to single molecules corresponding to the N-terminal 480 amino acids of FHA (which initiate outer membrane translocation) and observe equilibrium unfolding and refolding in multiple discrete steps. This distributed (rather than cooperative) folding of isolated FHA provides evidence for processive, vectorial folding in vivo.

2312-Pos Board B82

Elucidating the Alpha-Synuclein Fibril Fold by Pulsed EPR

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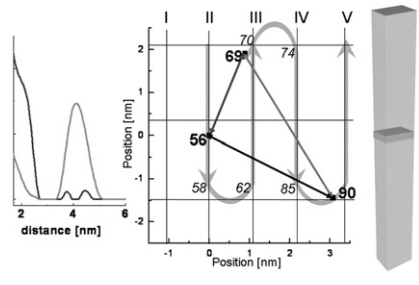
Amyloid fibrils are constituents of the plaques that are the hallmarks of neurogdegenerative diseases. In Parkinson's disease, these plaques (Lewy bodies) consist predominantly of the α-synuclein (αS) protein. To understand and interfere with aggregation, the structure of the fibrils (right Fig., green) needs to be known. Here we study the molecular architecture of the fibrils of αS by measuring distances between pairs of residues in the protein using double electron-electron paramagnetic resonance (DEER). Site-specific spin labeling was employed to create nine doubly labeled αS-variants that were investigated in the fibrillar state. Diamagnetic dilution with wild-type αS suppressed intermolecular interactions. The intramolecular distances provide constraints for the fold of the protein inside the fibril. Intramolecular distances were unambiguously observed for four pairs (41/69, 56/69, 56/90 and 69/90). Three of these distances (arrows) provide the constraints to suggest a model for the fold between residues 56 and 90 in the fibril (light blue). Assuming that only parallel β-sheets occur (l.-blue arrows), a model of four adjacent β-strands results (II-V), in which the strands comprise of eight to twelve residues each.



2313-Pos Board B83

Small Molecule Induced Conversion of Toxic Oligomers to Non-Toxic Beta-Sheet-Rich Amyloid Fibrils

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Several lines of evidence indicate that pre-fibrillar assemblies of amyloidogenic proteins such as soluble oligomers or protofibrils rather than mature, end-stage